GENE REPORTER ASSAY, KIT, AND CELLS FOR DETERMINING THE PRESENCE AND/OR THE LEVEL OF A MOLECULE THAT ACTIVATES SIGNAL TRANSDUCTION ACTIVITY OF A CELL SURFACE PROTEIN

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority from U.S. provisional application no. 60/415,818, filed October 4, 2002, the entire content of which is herein incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention relates to a gene reporter assay and a kit for determining the presence and/or the level in a sample of a molecule that activates the signal transduction activity of a cell surface protein. The present invention further relates to a cell which can be used in such an assay and to a method for preparing such a cell.

Description of the Related Art

[0003] Cell surface proteins permit intracellular transduction of extracellular signals. Cell surface proteins provide eukaryotic, as well as prokaryotic, cells a means to detect extracellular signals and transduce such signals intracellularly in a manner that ultimately results in a cellular response or a concerted tissue or organ response. Cell surface proteins, by intracellularly transmitting information regarding the extracellular environment via specific intracellular pathways induce an appropriate response to a particular stimulus. The response may be immediate and transient, slow and sustained, or some mixture thereof. By virtue of an array of varied membrane surface proteins, eukaryotic cells are exquisitely sensitive to their environment.

[0004] Extracellular signal molecules, such as cytokines, growth factors, hormones, vasodilators and neurotransmitters, exert their effects, at least in part, via interaction with cell surface proteins. For example, some extracellular signal molecules cause changes in transcription of target gene via changes in the levels of secondary messengers, such as cAMP. Other signals, indirectly alter gene expression by activating the expression of genes, such as immediate-early genes that encode regulatory proteins, which in turn activate expression of other genes that encode transcriptional regulatory proteins. Other extracellular signal molecules cause activation of latent cytoplasmic signal transducers and activators of transcription (STAT) protein that enhance the transcription of specific sets of genes.

[0005] Cell surface receptors and ion channels are among the cell surface proteins that respond to extracellular signals and initiate the events that lead to this varied gene expression and response. Ion channels and cell surface-localized receptors are ubiquitous and physiologically important cell surface membrane proteins. They play a central role in regulating intracellular levels of various ions and chemicals, many of which are important for cell viability and function.

Cell Surface Receptors

[0006] Cell surface-localized receptors are membrane spanning proteins that bind extracellular signalling molecules or changes in the extracellular environment and transmit the signal via signal transduction pathways to effect a cellular response. Cell surface receptors bind circulating signal molecules, such as cytokines, growth factors and hormones, etc., as the initiating step in the induction of numerous intracellular pathways. Receptors are classified on a structural basis or on the basis of the particular type of pathway that is induced. Among these classes of receptors are classes of cytokine receptors which include those that bind growth factors and have intrinsic tyrosine kinase activity, such as the heparin binding growth factor (HBGF) receptors, the immunoglobulin receptor superfamily, the hematopoietin/cytokine receptor superfamily, the nerve-growth factor receptor superfamily, other receptor tyrosine or serine kinases, and those that couple to effector proteins through guanine nucleotide binding regulatory proteins, which are referred to as G protein coupled receptors and G proteins, respectively.

[0007] Cytokines are intercellular messengers which coordinate communication between cells within a particular tissue, for example, antibody and T cell immune system interactions, and serve to modulate or modify the biological response. They are pleiotropic and have a broad spectrum of biological effects on more than one type of cell or tissue. The receptors for cytokines are broadly grouped into two classes, where the Class I cytokine receptors include receptors that bind various interleukins (IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15), erythropoietin (EPO), growth hormone (GH), granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF), and the Class II cytokine receptors include receptors that bind interferon (IFN) α/β, IFNγ, and IL-10.

Interferon receptors

[0008] Human interferons (IFNs) are a family of homologous helical cytokines composed of four distinct species: α, β, γ and ω based on nucleotide and amino acid sequence homology. The Type I IFNs, α, β, and ω, are encoded by at least 12 functional IFNα genes, an IFNω gene, and a more distantly related IFNβ gene. Type

II IFN, or IFNγ, is encoded by an unrelated gene and binds to a distinct cell surface receptor (De Maeyer et al., 1988; Pestka et al., 1987 and Diaz et al., 1993).

[0009] Type I IFNs bind to a common receptor, as shown by their ability to cross-compete for receptor binding (Pestka et al., 1987; Branca et al., 1981; and Merlin et al., 1985). The Type 1 interferon receptor has the largest number of natural ligands, some 14 in all, of all known cytokine receptors. Binding of interferons to their cell surface receptor represents the initial and probably most specific step in the IFN signaling pathway.

[0010] The Type I IFN receptor is composed of two transmembrane glycoproteins, IFNAR1 and IFNAR2 (Uze et al., 1990; Novick et al., 1994; Lutfalla et al., 1995; Domanski et al., 1995), which are rapidly tyrosine-phosphorylated following IFN binding (Platanias et al., 1994; Constantinescu et al., 1994; and Abramovich et al., 1994). Both subunits belong to the class II cytokine receptor superfamily (Bazan et al., 1990 and Thoreau et al., 1990) and are required for high affinity ligand binding and the establishment of biological activity (Langer et al., 1996 and Domanski et al., 1996). Class II cytokine receptors are distinguished from Class I receptors on the basis of the patterrn of the conserved pairs of cysteine residues that are thought to form disulfide bonds.

[0011] In contrast to other cytokine receptors, particularly the IFN-γ receptor, neither IFNAR1 nor IFNAR2 alone bind to IFNα or IFNβ with an affinity comparable to the heterodimer. Despite the fact that IFNAR2 plays a prominent role in ligand binding, IFNAR1 contributes to IFN binding by increasing the affinity of the receptor complex (4-10 fold) relative to that of IFNAR2 alone. IFNAR1 also modulates the specificity of ligand binding relative to that observed with IFNAR2 alone (Cohen et al., 1995; Russell-Harde et al., 1995; Cutrone et al., 1997; and Cook et al., 1996). IFNAR1 has a larger extracellular domain than most other class II cytokine receptors, composed of 4 immunoglobulin-like subdomains separated by di- or tri-proline motifs which can be divided into two tandem repeats (Novick et al., 1994; Lutfalla et al., 1992; and Uzé et al., 1995).

[0012] Human, murine and bovine IFNAR1 have been cloned and expressed in human and murine cells. Studies performed with transfected cells show that IFNAR1 plays a central role in ligand binding, cellular responses to IFNs and in the induction of the biological activities of the Type I interferons (Novick et al., 1994; Abramovich et al., 1994; Uzé et al., 1992; Mouchel-Vielh et al., 1992; Lim et al., 1993; Cleary et al., 1994; Constantinescu et al., 1995; Hwang et al., 1995; Vandenbroek et al., 1995; and Colamonici et al., 1994). Furthermore, the intracellular domain of IFNAR1 has been shown to play a key role in the transduction of the signal initiated at the cell surface by binding of Type I interferons to the nucleus (Basu et al., 1998). Targeted disruption of the IFNAR1 gene results in the loss of the antiviral response to Type I IFNs demonstrating that this receptor polypeptide is an essential component of the receptor complex and that both IFNAR1 and IFNAR2 subunits are required for IFNα and IFNβ signaling (Vandenbroek et al., 1995; Muller et al., 1994; Fiette et al., 1995; Steinhoff et al., 1995; and van den Broek et al., 1995).

[0013] Binding of type I interferon to the receptor complex activates two Janus kinases, Tyk2 and JAK1, which mediate the tyrosine phosphorylation and activation of two latent cytoplasmic transcription factors STAT1 and STAT2 which form a complex with a p48 DNA binding protein, interferon responsive protein 9 (IRF 9), which is translocated to the nucleus to promote specific gene transcription (Fu et al., 1992; Schindler et al., 1992; Darnell et al., 1994; Ihle et al, 1995; and Taniguchi, 1995). Both Tyk2 and STAT2 are constitutively associated with the membrane proximal region of the IFNAR1 chain, while JAK1 and STAT1 are physically associated with IFNAR2 and all four factors are rapidly activated during IFNα stimulation (Lutfalla et al., 1995; Bazan, 1990; Basu et al., 1998; Barbieri et al., 1994; Velazquez et al., 1995; Uddin et al., 1995; Yan et al., 1996(a) and 1996(b).

G-coupled receptors

[0014] The G protein transmembrane signaling pathways consist of three proteins: receptors, G proteins and effectors. G proteins, which are the intermediaries in transmembrane signaling pathways, are heterodimers and consist of α, β and γ subunits. Among the members of a family of G proteins the α subunits differ. Functions of G proteins are regulated by the cyclic association of GTP with the α subunit followed by hydrolysis of GTP to GDP and dissociation of GDP.

[0015] G protein coupled receptors are a diverse class of receptors that mediate signal transduction by binding to G proteins. Signal transduction is initiated via ligand binding to the cell membrane receptor, which stimulates binding of the receptor to the G protein. The receptor G protein interaction releases GDP, which is specifically bound to the G protein, and permits the binding of GTP, which activates the G protein. Activated G protein dissociates from the receptor and activates the effector protein, which regulates the intracellular levels of specific second messengers. Examples of such effector proteins include adenyl cyclase, guanyl cyclase, phospholipase C, and others.

Growth Factors and Growth Factor Receptors

[0016] Polypeptide growth factors are modulators of cell proliferation and differentiation whose biological functions are mediated by the interaction of the growth factor with cell surface receptors and subsequent alterations in gene expression. Growth factors bind to specific receptors and appear to induce tyrosine phosphorylation and c-fos mRNA synthesis. In addition, at least some growth factors, such as platelet-derived growth factor (Yeh et al., 1987) and heparin-binding growth factor-2 or basic fibroblast growth factor (Bouche et al., 1987), are translocated to the nucleus.

[0017] Activation of growth factor receptors by interaction with specific growth factors or with agents such as phorbol mistric acetate (PMA) activates protein kinase C, which is a family of phospholipid- and calcium-activated protein kinases. This activation results in the transcription of an array of proto-oncogene transcription factor encoding genes, including c-fos, c-myc and c-jun, proteases, protease inhibitors, including collagenase type I and plasminogen activator inhibitor, and adhesion molecules, including intercellular adhesion molecule I. Protein kinase C activation antagonizes growth factor activity by the rapid phosphorylation of growth factor receptors, which thereby decreases tyrosine kinase activity. Growth factors and other mitogens that induce cell proliferation and cell growth are believed to play a role in tumor growth, which often carry identifiable cell surface receptors specific for growth factors and other extracellular signals.

[0018] The interaction of nerve growth factor (NGF) with its receptor is typical of the array of responses such an extracellular signal induces. NGF is a polypeptide growth hormone that is necessary for differentiation and growth of the neural crest-derived sensory neuron. NGF binds to its specific cell surface receptor and is retrogradely transported to the cell body (Changelian et al., 1989). This initiates a cascade of intracellular events, culminating in a differentiated phenotype. PC12 cells, which are a rat pheochromocytoma cell line, are used as a model for the study of NGF-mediated differentiation. When treated with NGF, PC12 cells change from replicating adrenal-chromaffin-like cells to nonreplicating, electrically excitable sympathetic-neuron-like cells.

[0019] Concomitant with the phenotypic changes, there is induction and expression of specific genes. Binding of NGF to PC12 cells induces the immediate and rapid expression of certain genes, including the c-fos, NGF1-A and NGF1-B genes, which are referred to as early genes. Such early genes are believed to encode transcriptional regulators. The NGF-1A gene product contains tandemly repeated "zinc finger" domains that are characteristic of DNA-binding proteins, and the NGF1-B protein is homologous to members of the glucocorticoid receptor family and, thus, may function as a ligand-dependent modulator of transcription. The c-fos gene product, FOS appears to function as a transcriptional regulatory molecule.

The c-fos Gene and Related Genes

[0020] As discussed above, induction of expression of the c-fos gene is an event that is common to a number of response pathways that are initiated by the activity of a variety of cell surface proteins.

[0021] The c-fos gene product, FOS, associates with the transcription activator JUN, which is the product of the c-jun gene, to form a complex that forms a transcription activation complex, AP-1. Transcription of both c-fos and c-jun is induced rapidly and transiently following stimulation. The induced mRNAs accumulate for 1-2 hours in the cytoplasm where the FOS and JUN proteins, which are short-lived, are translated and then translocated to the nucleus to form a heterodimeric protein complex that binds to the DNA regulatory element, AP-1 binding site.

[0022] The c-fos and c-jun genes are members of gene families that encode proteins that participate in the formation of heterodimeric complexes that interact with AP-1 binding sites. Transcription factor AP-1 is composed of several protein complexes whose concentrations change upon cell stimulation. These complexes specifically interact with a seven-base core nucleotide sequence motif, that is known to be a relatively common constituent of both positive and negative transcriptional regulatory elements and that is required for both basal and induced levels of gene expression.

[0023] The gene products, FOS and JUN cooperate in the regulation of target genes that underlie many cellular and adaptive responses to the environment. They are involved in a number of neurophysiological processes.

[0024] Thus, c-fos induction involves distinct second messenger pathways that act via separate regulatory elements and that differentially modify, the resulting gene product, FOS, which in turn interacts in different ways with differentially modified JUN protein. Therefore, a multitude of extracellular events induce expression of a small number of inducible proteins that form an array of protein complexes that can differentially bind to DNA regulatory elements that contain AP-1 binding sites. Therefore, numerous cell surface proteins can act via overlapping transduction pathways and transduce extracellular signals that ultimately induce a variety of responses.

[0025] There are many assays that may rely on *in vivo* activity in a living cell line. One example is a cell line having an Interferon Stimulatory Response Element (ISRE) connected to a luciferase gene, or another reporter gene, so that when the cell line is subjected to the presence of interferon as an extracellular signal, the signal transduction activity of endogenous interferon cell surface receptors produces a signal that activates the ISRE, which then causes transcription of the luciferase gene. Thus, the activity of luciferase in creating light can be measured and is related to the amount of interferon which is present in the sample, and which is proportional to the amount of interferon over a particular range (Lallemand et al., 1996).

[0026] Lleonart et al. (1990) described a reporter gene assay for Type I interferon based on monkey Vero cells transfected with Type I interferon inducible mouse Mx promoter linked to the human growth hormone (hGH) gene as the reporter gene. This Type I interferon assay was developed further by transfecting monkey Vero cells with a plasmid carrying the luciferase reporter gene under the control of the Type I interferon inducible mouse Mx1 promoter (Canosi et al., 1996).

[0027] A further type of interferon reporter gene assay was developed by Hammerling et al. (1998) who used a human glioblastoma cell line transfected with a reporter gene construct of glial fibrillary acidic protein (GFAP) promoter and an *E. coli* β-galactosidase (lacZ) reporter gene. In this particular assay, it is the reduction/inhibition of β-galactosidase expression by either human Type I or Type II interferon in a selective and dose dependent manner that is measured.

[0028] With the types of cell lines which can be used in such assays, there is a problem in commercializing them in that once the cell line is shipped for use in a single assay, the end user can grow the cells and then use their own stock of such cells for future assays without the need to order more cells from the supplier.

[0029] Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on the information available to applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

[0030] It is an object of the present invention to develop a way to make a desirable cell line an essentially "one time use" commercial cell line with a sufficiently long shelf life for its intended purpose so that whenever a user uses this commercial cell line, such as in an assay, this user must purchase cells of this commercial cell line from a supplier.

[0031] Thus, the present invention provides a cell transformed with a reporter gene construct in which the expression of the reporter gene product is regulated by the signal transduction activity of a cell surface protein in response to an extracellular signal. This cell according to the present invention has been treated so that it will maintain signal transduction activity for at least about 1 hour, but no more than 30 days at a temperature above freezing, before losing that ability. Such a treated cell has acquired the commercial advantage of having a sufficiently long shelf life for its purpose by maintaining the cell in a state or by inhibiting cell division, whereupon at the end of its useful shelf life or at the end of its use the cell undergoes cellular death such as by apoptosis.

[0032] The present invention also provides a cell based assay kit for determining the level in a sample of a molecule that activates the signal transduction activity of a cell surface protein. Such a kit includes a testing device with multiple wells and a reagent containing a plurality of the cell of the present invention.

[0033] Further provided by the present invention is a method for preparing the cell of the present invention, which loses signal transduction within 30 days. This method involves transforming a host cell with a reporter gene construct described above for the cell of the present invention and then treating the transformed cell so that the treated transformed cell will maintain signal transduction activity for at least about 1 hour but no more than about 30 days at a temperature above freezing before losing the signal transduction activity.

[0034] Another aspect of the present invention is directed to a method for determining the presence and/or the level in a sample of a molecule that activates the signal transduction activity of a cell surface protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] Figure 1 shows a schematic representation of a luciferase reporter gene construct where luciferase expression is under the control of a chimeric promoter containing an interferon sensitive response element (ISRE) from the ISG15 gene and a minimal SV40 promoter.

[0036] Figure 2 shows a schematic representation of an enhanced green fluorescent protein (EGFP-1) reporter gene construct where EGFP-1 expression is under the control of a chimeric promoter containing an ISRE from the ISG15 gene and a minimal SV40 promoter.

[0037] Figure 3 shows the standard curves of luciferase activity in PIL5 cells in the presence of different amounts of each of the various isotypes of human Type I interferon.

[0038] Figure 4 is a graph showing luciferase activity in PIL5 cells four days after different doses of γ radiation and incubation at 37°C.

[0039] Figure 5 is a graph showing the percentage of viable PIL5 cells at various times after 6 Grays (Gy) of γ radiation and incubation at 37°C.

[0040] Figure 6 is a graph showing luciferase activity in PIL5 cells at various times after 6 Gy of γ radiation and incubation at room temperature.

[0041] Figure 7 is a graph showing luciferase activity in PIL5 cells eight days after 6 Gy of γ radiation and incubation at 4°C or room temperature.

[0042] Figure 8 is a graph showing luciferase activity in PIL5 cells five days after 6 Gy of γ radiation and incubation at 37°C in culture medium containing 0%, 1%, or 10% fetal bovine serum (FBS).

[0043] Figure 9 is a graph showing luciferase activity in different cell concentrations of PIL5 cells thirteen days after 6 Gy of γ radiation and incubation at 37°C in culture medium containing 10% or 20% fetal bovine serum (FBS).

[0044] Figure 10 is a graph showing luciferase activity in PIL5 cells fourteen days after 6 Gy of γ radiation and treatment with aurintricarboxylic acid at room temperature.

[0045] Figure 11 is a graph showing luciferase activity in PIL5 cells 24 hours after thawing from 1 month storage at -20°C in 10% DMSO. The PIL5 were treated for 1 hour with different concentrations of anti-mitotic and pro-apoptotic agents, vinblastine (Vin) or 5-fluorouracil (5Fu), prior to freezing and storage at -20°C.

[0046] Figure 12 is a graph showing the effect of vinblastine or 5-fluorouracil on interferon sensitivity of frozen cells. PIL5 cells were treated for 1 hour at 37°C with 1.0, 10, or 100 μM vinblastine, or 10, 100 μM, or 1.0 mM 5-fluorouracil, centrifuged, and suspended in 50 μl of RPMI 1640 medium with 20% fetal bovine serum (FBS) and 10% dimethlysulfoxide (DMSO), and $2x10^5$ cells were distributed into each well of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0047] Figure 13 is a graph showing the effect of vinblastine on interferon sensitivity of frozen cells. PIL5 cells were treated for 10 minutes at 37^0C with 0.1, or 1.0 μg/ml of vinblastine, centrifuged, and suspended in 25 μl of RPMI 1640 medium with 40% FBS and 10 % glycerol, and $2x10^5$ cells were distributed into each well of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0048] Figure 14 is a graph showing the effect of time of treatment with vinblastine on interferon sensitivity of frozen cells. PIL5 cells were treated for 10, 20, 30, or 40 minutes at 37^0C with 1.0 μg/ml vinblastine, centrifuged, and suspended in 50 μl of RPMI 1640 medium with 20% FBS and 10% DMSO, and $2x10^5$ cells were distributed into each well of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0049] Figure 15 is a graph showing the effect of glycerol concentration on interferon sensitivity of frozen cells. PIL5 cells were treated for 10 minutes at 37°C with 100 μM vinblastine, centrifuged, and suspended in 25 μl of RPMI 1640 medium with 40% FBS and 5, or 10% glycerol, and $2x10^5$ cells were distributed into each well of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0050] Figure 16 is a graph showing the effect of glycerol concentration on the interferon sensitivity of frozen cells. PIL5 cells were treated for 10 minutes at 37°C with 100 μM vinblastine, centrifuged, and suspended in 25 μl of RPMI 1640 medium with 40% FBS and 5, 10, 15, 20% glycerol, or 5% glycerol and 5% DMSO and $2x10^5$ cells were distributed into each well of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0051] Figure 17 is a graph showing the effect of glycerol in the presence or absence of DMSO on the interferon sensitivity of frozen cells. PIL5 cells were treated for 10 minutes at 37°C with 100 μM vinblastine, centrifuged, and suspended in 25 μl of RPMI 1640 medium with 40% FBS and 10% glycerol, with or without 2.5% DMSO, and $2x10^5$ cells were distributed into each well of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0052] Figure 18 is a graph showing the effect of cell density on the interferon sensitivity of frozen cells. PIL5 cells were treated for 10 minutes at 37°C with 100 μM vinblastine, centrifuged, and $2x10^5$ cells suspended in 10, 25, or 50 μl of RPMI 1640 medium with 40% FBS and 10% DMSO, and distributed into the wells of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0053] Figure 19 is a graph showing the effect of cell density on the interferon sensitivity of frozen cells. PIL5 cells were treated for 10 minutes at 37°C with 100 μM vinblastine, centrifuged, and 2×10^5 cells suspended in 25 or 50 μl of RPMI 1640 medium with 40% FBS and 10% DMSO, and distributed into the wells of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0054] Figure 20 is a graph showing the effect of cell density on the interferon sensitivity of frozen cells. PIL5 cells were treated for 10 minutes at 37°C with 100 μM vinblastine, centrifuged, and 2×10^5 cells suspended in 10, 25, or 50 μl of RPMI 1640 medium with 40% FBS and 10% glycerol, and distributed into the wells of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0055] Figure 21 is a graph showing the effect of cell number on interferon sensitivity following freezing. PIL5 cells were treated for 10 minutes at 37°C with 100 μM vinblastine, centrifuged, and 0.5, 1.0, or $2x10^5$ cells suspended in 10 μl of RPMI 1640 medium with 40% FBS and 10% glycerol, and distributed into the wells of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0056] Figure 22 is a graph showing the effect of cell number on interferon sensitivity following freezing. PIL5 cells were treated for 10 minutes at 37°C with 100 μM vinblastine, centrifuged, and 0.5, 1.0, or $2x10^5$ cells suspended in 10 μl of 40% FBS and distributed into the wells of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

[0057] Figure 23 is a graph showing the effect of freezing in a cryo-preservation ampoule or micro-titer plate on interferon sensitivity. PIL5 cells were treated for 10 minutes at 37°C with 1.0 μg/ml of vinblastine, centrifuged, and suspended of RPMI 1640 medium with 40% FBS, 10% glycerol, and 2.5% DMSO. The cells were then distributed into each well of a micro-titer culture plate

($2x10^5$ cells in 25 µl/well) or added to a cryo-preservation ampoule ($2x10^7$ cells in 1.0 ml) and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the micro-titer plate and cryo-preservation ampoule were thawed rapidly and a standard preparation of human IFN α was titrated in the micro-titer plate, or the contents of the cryo-preservation ampoule ($2x10^7$ cells in 1.0 ml) were diluted in 6.5 ml of RPMI 1640 medium and 75 µl of cell suspension was then distributed into each well of a micro-titer culture plate and a standard preparation of human IFN α was titrated in the micro-titer plate in the luciferase gene reporter assay procedure described above.

[0058] Figure 24 is a graph showing the titration of a standard interferon preparation on PIL5 cells following freezing. PIL5 cells were treated for 10 minutes at 37°C with 1.0 µg/ml of vinblastine, centrifuged, and suspended at a concentration of $2x10^5$ cells/25 µl in RPMI 1640 medium with 40% FBS, 10% glycerol, and 2.5% DMSO, and distributed into each well of a micro-titer culture plate and frozen at -80°C. After storage at -80°C and subsequent storage at -20°C, the plate was thawed rapidly and a standard preparation of human IFN α was titrated in the luciferase gene reporter assay procedure described above.

DETAILED DESCRIPTION OF THE INVENTION

[0059] The present invention is directed to a cell transformed with a reporter gene construct comprising a nuclotide sequence encoding a reporter gene product operatively linked to one or more transcriptional control elements that is regulated by the signal transduction activity of a cell surface protein in response to an extracellular signal. This cell of the present invention has been treated in such a way that it will maintain the signal transduction activity of the cell surface protein for at least about 1 hour but no more than about 30 days at a temperature above freezing before losing the signal transduction activity. Thus, not only does the cell of the present invention have a sufficient shelf life by inhibiting cell division or by maintaining the cell in a frozen state for the purpose desired by an end user, such as for conducting an assay, but it has been treated in such a manner that the cells can be frozen, or even kept at room temperature, allowing storage for extended periods and transportation in a frozen state or at room temperature. The cell of the present invention also has the commercial advantage to a supplier of being a one time use cell that cannot be propagated by the end user for possible further use. Instead, the cell, preferably as part of a kit, must be purchased from the supplier for each single use.

[0060] The cell according to the present invention may be any eukaryotic or prokaryotic cell. Mammalian and avian cells are however preferred, with human cells most preferred. Non-limiting examples of other suitable cells include other vertebrate cells, plant protoplasts, fungal and yeast cells, and bacterial cells.

[0061] The cell surface protein from which its signal transduction activity, in response to an extracellular signal, regulates the expression of a reporter gene product can be any cell surface protein that is known to those of skill in the art or that may be identified by those of skill in the art. Exemplary cell surface proteins include, but are not limited to, cell surface receptors and ion channels. Non-limiting examples of cell surface receptors include cytokine receptors (e.g., receptors for Type I interferon, Type II interferon, interleukins, growth hormone, erythropoietin (EPO), granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), etc.), growth factor receptors, hormone receptors, T cell receptors, antigen receptors, complement receptors, and neuroreceptors. The reference text, J. M. Cruse and Robert E. Lewis, Atlas of Immunology, CRC Press, Washington, DC, 1999, which discloses many receptors involved in immune response and immune system interactions is entirely incorporated herein by reference. Cell surface receptors also include, but are not limited to, muscarinic receptors (e.g., human M2 (GenBank accession #M16404); rat M3 (GenBank accession #M16407); human M4 (GenBank accession #M16405); human M5 (Bonner et al., 1988); and the like); neuronal nicotinic acetylcholine receptors (e.g., the $\alpha2$, $\alpha3$ and $\beta2$ subtypes); the rat $\alpha2$ subunit (Wada et al., 1988); the rat $\alpha3$ subunit (Boulter et al., 1986); the rat $\alpha4$ subunit (Goldman et al., 1987); the rat $\alpha5$ subunit (Boulter et al., 1990); the rat $\beta2$ subunit (Deneris et al., 1988); the rat $\beta3$ subunit (Deneris et al., 1989); the rat $\beta4$ subunit (Duvoisin et al., 1989); combinations of the rat α subunits, β subunits and α and β subunits; GABA receptors (e.g., the bovine $\alpha1$ and $\beta1$ subunits (Schofield et al., 1987); the bovine $\alpha2$ and $\alpha3$ subunits (Levitan et al., 1988); the γ-subunit (Pritchett et al., 1989); the $\beta2$ and $\beta3$ subunits (Ymer et al., 1989); the δ subunit (Shivers, B.D., 1989); and the like); glutamate receptors (e.g., receptor isolated from rat brain (Hollmann et al., 1989); and the like); adrenergic receptors (e.g., human $\beta1$ (Frielle et al., 1987); human $\alpha2$ (Kobilka et al., 1987); hamster $\beta2$ (Dixon et al., 1986); and the like); dopamine receptors (e.g., human D2 (Stormann et al., 1990); rat (Bunzow et al., 1988); and the like); NGF receptors (e.g., human NGF receptors (Johnson et al., 1986); and the like); serotonin receptors (e.g., human 5HT1a (Kobilka et al., 1987); rat 5HT2 (Julius et al., 1990); rat 5HTlc (Julius et al., 1988); and the like).

[0062] Ion channels include, but are not limited to, calcium ion channels (e.g., human neuronal α2 subunit (see W089/09834); rabbit skeletal muscle α1 subunit (Tanabe et al. 1987); rabbit skeletal muscle α2 subunit (Ellis et al., 1988); rabbit skeletal muscle β subunit (Ruth et al., 1989); rabbit skeletal muscle γ subunit (Jay et al., 1990); and the like); potassium ion channels (e.g., rat brain (BK2) (McKinnon, D., 1989); mouse brain (BK1) (Tempel et al., 1988); and the like); sodium ion channels (e.g., rat brain I and II (Noda et al., 1986); rat brain III (Kayano et al., 1988); and others).

[0063] It will be appreciated by those of skill in the art that the cell surface protein discussed above is preferably endogenous to the cell of the present invention. However, it will also be appreciated that the cell surface protein may be expressed from cloned DNA, such as to supplement the number of the cell surface protein at the surface of the cell, or the cell surface protein may be expressed from cloned DNA but is a cell surface protein that is heterologous to the host cell.

[0064] For signal transduction, the intracellular signal that is transduced is initiated by the specific interaction of an extracellular signal, i.e., a molecule or a change in environment, with a receptor or ion channel present on the cell surface. This interaction sets in motion a cascade of intracellular events, the ultimate consequence of which is a rapid and detectable change in the expression of a gene product, which in the cell of the present invention is a reporter gene product. The extracellular signal or effector molecule is any compound or substance that in some manner specifically alters the activity of a cell surface protein. Examples of such signals include, but are not limited to, molecules such as cytokines (i.e., interferons), growth factors, hormones, endorphins, neurotransmitters, acetylcholine, and mitogenic substances, such as phorbol mistric acetate (PMA), that bind to cell surface receptors and ion channels and modulate the activity of such receptors and channels. For example, antagonists are extracellular signals that block or decrease the activity of cell surface protein and agonists are examples of extracellular signals that potentiate, induce or otherwise enhance the activity of cell surface proteins.

[0065] The reporter gene construct carried by the cell of the present invention is a DNA molecule that includes a nucleotide sequence encoding a reporter gene product operatively linked to transcriptional control elements/sequences. Transcription of the reporter gene is controlled by these sequences. The activity of at least one or more of these control sequences is directly or indirectly regulated by the cell surface protein. The transcriptional control sequences include but are not limited to promoters and other regulatory regions, such as enhancer sequences and repressor and activator binding sites, that modulate the activity of the promoter, or control sequences that modulate the activity or efficiency of the RNA polymerase that recognizes the promoter, or control sequences that are recognized by effector molecules, including those that are specifically induced by interaction of an extracellular signal with a cell surface protein. For example, modulation of the activity of the promoter may be effected by altering the RNA polymerase binding to the promoter region, or, alternatively, by interfering with initiation of transcription or elongation of the mRNA. Such sequences are herein collectively referred to as transcriptional control elements or sequences. In addition, the construct may include sequences of nucleotides that alter translation of the resulting mRNA, thereby altering the amount of reporter gene product expressed.

[0066] A promoter that is regulated or mediated by the activity of a cell surface protein is a promoter whose activity changes when a cell is exposed to a particular extracellular signal by virtue of the presence of cell surface proteins whose activities are affected by the extracellular signal. For example, the c-fos promoter is specifically activated upon the specific interaction of certain extracellular signals, such as growth hormones, with a cell surface protein, such as a growth hormone receptor. In particular, the regulation of such promoters by the cell surface protein, though indirect, occurs within minutes of the interaction of the cell surface protein with the extracellular signal. As used herein, operative linkage refers to the linkage of a transcriptional control element, i.e., promoter, to a nucleotide coding sequence such that the transcriptional control element is properly positioned for its activity of binding RNA polymerase and initiating transcription of the nucleotide coding sequence. Thus, a nucleotide coding sequence in operative linkage with a promoter is downstream, with respect to the direction of transcription, from the promoter, is in the correct reading frame with respect to the transcription initiation site and is inserted in a manner such that transcription elongation proceeds through the nucleotide coding sequence.

[0067] Suitable transcriptional control elements may be obtained or derived from the transcriptional regulatory regions of genes whose expression is rapidly induced, generally within minutes, of contact between the cell surface protein and the effector protein that modulates the activity of the cell surface protein. Examples of such genes include, but are not limited to, the immediate early genes (Sheng et al., 1990), such as c-fos. Immediate early genes are genes that are rapidly induced upon binding of a ligand to a cell surface protein. The transcriptional control elements that are preferred for use in the reporter gene constructs include transcriptional control elements from immediate early genes, elements derived from other genes that exhibit some or all of the characteristics of the immediate early genes, or synthetic elements that are constructed such that genes in operative linkage therewith exhibit such characteristics. The characteristics of preferred genes from which the transcriptional control elements are derived include, but are not limited to, low or undetectable expression in quiescent cells, rapid induction at the transcriptional level within minutes of extracellular simulation, induction that is transient and independent of new protein synthesis, subsequent shut-off of transcription requires new protein synthesis, and mRNAs transcribed from these genes have a short half-life. It is not necessary for all of these properties to be present.

[0068] Suitable promoters and transcriptional control elements include, but are not limited to, the vasoactive intestinal peptide (VIP) gene promoter (cAMP responsive; Fink et al., 1988); the somatostatin gene promoter (cAMP responsive; Montminy et al., 1986); the proenkephalin promoter (responsive to cAMP, nicotinic agonists, and phorbol esters; Comb et al. 1986); the phosphoenolpyruvate carboxy-kinase gene promoter (cAMP responsive; Short et al., 1986); the NGFI-A gene promoter (responsive to NGF, cAMP, and serum; Changelian et al., 1989); the transcriptional control elements obtained or derived from the c-fos gene; and others that may be known to or prepared by those of skill in the art.

[0069] The c-fos proto oncogene is the cellular homologue of the transforming gene of FBJ osteosarcoma virus. It encodes a nuclear protein that is most likely involved in normal cellular growth and differentiation. Transcription of c-fos is transiently and rapidly activated by growth factors and by inducers of other cell surface proteins, including hormones, differentiation-specific agents, stress, mitogens and other known inducers of cell surface proteins. Activation is protein synthesis independent. The c-fos regulatory elements include a TATA box that is required for transcription initiation, two upstream elements for basal transcription, and an enhancer, which includes an element with dyad symmetry and which is required for induction by TPA, serum, EGF, and PMA. The 20 bp transcriptional enhancer element located between -317 and -298 bp upstream from the c-fos mRNA cap site, which is essential for serum induction in serum starved NIH 3T3 cells. One of the two upstream elements is located at -63 to -57 and it resembles the consensus sequence for cAMP regulation.

[0070] Transcriptional control elements, particularly as they relate to a preferred embodiment of the present invention where

Type I and/or Type II interferon is the extracellular signal, are preferably an interferon stimulatory response element (ISRE) and/or a gamma activated sequence (GAS). There are a number of ISREs characterized from different human genes responsive to Type I interferon and a consensus sequence, ggraaagwGAAActg (SEQ ID NO:6; capital letters denote core sequence; underlines denote high conservation), to which the STAT1/STAT2/IRF9 complex binds, was identified for ISRE (Levy et al., 1988). A preferred ISRE is from the human ISG15 gene and is presented as SEQ ID NO:5 where nucleotides 41-55 correspond to the consensus ISRE sequence. ISRE is also highly conserved among species. For example, a sequence present in the promoter region of the interferon inducible chicken Mx gene (Schumacher et al., 1994) is similar to that found in primates and conforms to the ISRE consensus sequence for mammalian interferon responsive genes including rodents and cows (see Fig. 2 of Perry et al., 1999).

[0071] Regarding GAS, to which the STAT1 homodimer binds in genes responsive to Type II interferon, a consensus sequence, nnnsanttccgGGAAntgnsn (SEQ ID NO:7; capital letters denote core sequence; underlines denote high conservation), from many selected binding sequences was identified (Horvath et al., 1995).

[0072] In the embodiment of the present invention where Type I interferon (see Example presented hereinbelow) and/or Type II interferon is the extracellular signal, a preferred combination of transcriptional control elements is an interferon responsive chimeric promoter in which an ISRE and/or GAS controls a SV40 minimal promoter operatively linked to a nucleotide sequence encoding a reporter gene product.

[0073] The reporter gene product, whose level is a measure of the presence and/or the level of a molecule that activates the signal transduction activity of a cell surface protein, may be RNA or protein, as long as it is readily detectable. For instance, firefly luciferase, enhanced green fluorescent protein (EGFP) and jellyfish aequorin are most preferred embodiments of reporter gene products used according to the present invention. In the case of the enzyme firefly luciferase (deWet et al., 1987) and jellyfish aequorin (Rider et al., 2003), the result of its enzymatic activity, light, is detected and measured using a luminometer, whereas in the case of EGFP, a fluorescence activated cell sorter or analyzer (FACS) can be used at an appropriate wavelength to detect and quantify the amount of EGFP expressed in a cell. The distribution curve of the amount of luciferase, aequorin or EGFP expressed in a sample of cells will be determined by the amount of ligand (within a given range) to which the cell is exposed. Non-limiting examples of other suitable reporter gene products include dsRED, chloramphenicol acetyl transferase (CAT) (Alton et al., 1979) other enzyme detection systems, such as β-galactosidase, bacterial luciferase

(Engebrecht et al., 1984 and Baldwin et al. 1984), alkaline phosphatase (Toh et al. 1989 and Hall et al. 1983), and bacterial or humanized β-lactamase (Zlokarnik et al., 1998).

[0074] In order to provide the cell of the present invention, which is a one time use cell that cannot be propagated for further use, the cell transformed with a reporter gene construct is treated in such a way that it will maintain the signal transduction activity of the cell surface protein for at least about 1 hour but no more than about 30 days at a temperature above freezing before losing the signal transduction activity. Thus, according to one aspect of the present invention, which is a method for preparing a cell transformed with a reporter gene construct that loses signal transduction activity within about 30 days, a cell is transformed with a reporter gene construct containing a nucleotide sequence encoding a reporter gene product operatively linked to one or more transcriptional control elements that is regulated by the signal transduction activity of a cell surface protein in response to an extracellular signal. The transformed cell is then treated so that it will maintain the signal transduction activity of the cell surface protein for at least 1 hour but no more than about 30 days at a temperature above freezing before losing this activity.

[0075] One preferred embodiment of the present invention is where the transformed cell is treated by irradiating with γ-

radiation at an intensity and for a sufficient time such the irradiated cell maintains the signal transduction activity of the cell surface protein for a period of at least about 7 days but no more than 30 days at a temperature above freezing following irradiation, after which period of time the irradiated cell immediately undergoes cellular death (i.e., apoptosis).

[0076] It is known that γ-irradiation at a high dose causes a cell to lose its signal transduction activity. Irradiation at a somewhat lower dose causes a cell to cease replication and undergo cellular death. The present inventors have now discovered that it is possible to determine a dose which inhibits replication but still allows a cell to maintains its signal transduction activity for a period of time before undergoing cell death. For example, γ-irradiation at about 9 Grays allows a cell to retain signal transduction activity for 14 days, after which the cells undergo cell death. However, during those 14 days, the signal transduction activity in response to, for example, Type I interferon that is being assayed functions as well as in a non-irradiated control. This is shown in the experiments using a luciferase gene reporter assay for Type I interferon that are presented in the Example hereinbelow. Thus, by irradiating a cell with γ radiation, the present invention provides a treated cell with a 14-day shelf life, but which becomes inactive (undergoes cellular death) after a period of about 14 days so that it cannot be maintained and reproduced by an end user.

[0077] According to the irradiation embodiment of the present invention, the dose (intensity and duration) of γ radiation to which the transformed cell is treated is preferably about 6 to 12 Grays (Gy). As the experiments in the Example presented hereinbelow demonstrate, the temperature above freezing, at which the cell is kept or stored, affects the shelf-life of the cell. Preferably, this temperature is room temperature, which advantageously maintains maximum interferon sensitivity while providing for ease of storage and shipping of the commercial one time use cell of the present invention.

[0078] A second preferred embodiment of the present invention is where the transformed cell is treated with an anti-mitotic and pro-apoptotic agent such as vinblastine, 5-fluorouracil (5-Fu), or cisplatin in a sufficient amount and for a sufficient time such that the treated cell maintains the signal transduction activity of the cell surface protein for a period of at least about 1 hour but no more than about 30 days at a temperature above freezing following treatment with the agent, after which period of time the treated cell immediately undergoes cellular death. An anti-mitotic and pro-apoptotic agent will affect a treated cell when it begins to replicate, thereby inducing apoptosis and killing the cell. Thus, cells which have been treated with an anti-mitotic and pro-apoptotic agent, such as the human promonocytic cells transformed with a luciferase reporter gene construct exemplified in the Example hereinbelow, will have a shelf life of about 24 hours during which the signal transduction assay can be conducted and after which period of time the cells will die. It will be appreciated that a cell having only a 24 hour shelf life is not desirable from a commercial standpoint. In order to extend the shelf life, the treated cells may be immediately frozen, in which state they will have a much longer shelf life, depending upon the manner of freezing and thawing. Once thawed, however, they must be used within 24 hours, after which they will undergo cellular death (i.e., apoptosis).

[0079] It should be understood that conventional wisdom is that cryopreservation of cells requires a special freezing and thawing process (and equipment) in which the cells are frozen at a rate of 1°C per minute until it reaches -80°C or liquid nitrogen temperatures of about -200°C, where it may be stored indefinitely, and after which it must be thawed very rapidly. Often, dimethyl sulfoxide (DMSO) or another cryopreservative is also used in order to help protect the cells. If the cells are treated with an anti-mitotic and pro-apoptotic agent, they can be frozen with this cumbersome cryopreservation technique for an indefinite period of time and then be used for a purpose, such as a gene reporter assay for signal transduction activity, for 24 hours after being thawed. However, this is considered a less commercially viable technique as it would greatly increase the manufacturing/processing cost.

[0080] As most laboratories do not have storage facilities at -200°C or even -80°C, it would be useful to allow freezing of the cells to occur at -20°C. However, it is known that cell viability is poor when cells are frozen at -20°C and then thawed. In the course of the experimentation leading to the present invention, it was unexpectedly discovered by the present inventors that DMSO will protect the cells even when frozen at -20°C without any special freezing or thawing techniques or equipment. While glycerol, a known cryopreservative compound, will protect cells at -20°C, there is the possibility that it may prevent protein ligands from interacting with surface receptors at the high percentage (50%) of glycerol conventionally used for cryopresevation. However, a low percentage of glycerol (much less than the 50% conventionally used) can be used. DMSO does not have this disadvantage. It is a discovery of the present invention that DMSO will protect cells frozen at -20°C without any special freezing or thawing techniques or equipment being required and without adversely affecting their sensitivity to IFN as demonstrated in the Example hereinbelow (see Fig. 11). Thus, it is another surprising and inventive aspect of the present invention that after treating with an anti-mitotic and pre-apoptotic agent, a cell may achieve a long shelf life even at standard freezer temperatures of -20°C if further treated with DMSO and that once thawed such a cell will remain active, i.e., for signal transduction assays, for approximately 24 hours until it undergoes apoptosis as a result of being treated with an anti-mitotic and pro-apoptotic agent. Any anti-mitotic and pro-apoptotic agent which kills cells during the process of replication by inducing apoptosis, such as vinbastine, 5-FU and cisplatin, can be used for this purpose as it would be expected that the cells will remain biologically active during a quiescent period and until such time the treated cells start to die.

[0081] Thus, according to a second preferred embodiment of the present invention, the treated transformed cell is frozen at a temperature and under conditions such that it will resume signal transduction after thawing. While the cell is preferably frozen at a temperature between -20°C and -200°C, more preferably at –80°C, and subsequently stored at -20°C, a commonly available freezer temperature in almost all laboratories, it is intended that other suitable temperatures for cryopreservation of cells, such as the liquid nitrogen temperature of about -200°C, be encompassed by the present invention. It is further preferred that the treated transformed cell be resuspended in a solution containing a cryopreservative before freezing the cell. Dimethyl sulfoxide (DMSO) is the preferred cryopreservative although other suitable cryopreservatives which have a high bonding affinity to water, such as ethylene glycol, polyethylene glycol, propylene glycol, glycerol, butane diol, propanediol, and formamide, may be used so long as they do not interfere with the use of the cell after thawing. When DMSO is used alone as the cryopreservative, the solution containing DMSO preferably contains about 10% DMSO. More preferably, 2.5% DMSO is used in combination with 10% glycerol as the cryopreservative.

[0082] Another aspect of the present invention is directed to an assay kit for determining the level in a sample of a molecule that activates the signal transduction activity of a cell surface protein. This assay kit includes a plurality of the cell of the present invention (as a reagent) and a testing device having a plurality of wells. Preferably, the testing device is a multi-well microtiter plate, but can also be any type of receptacle, such as petri dishes or plates, with a plurality of wells in which an assay can be conducted to determine the level of a molecule in a sample. It is preferred that the cells as a component or reagent of the assay kit be disposed in the wells of the testing device, although it will be appreciated that such cells can instead be dispensed in the wells of the testing device by the end user just prior to conducting the assay. The kit may further include a set of instructions for using the kit to conduct the intended assay for determining the level of a molecule that activates the signal transduction activity in a sample.

[0083] The present invention further provides an assay method for determining the presence and/or the level in a sample, by reference to a standard included in the assay, of a molecule that activates the signal transduction activity of a cell surface protein, preferably a cell surface receptor. This assay method uses the cell of the present invention and can include the method of preparing such a cell according to the present invention as its initial step or steps. If the prepared cell is frozen according to a preferred embodiment of the present invention, then the cell must be thawed before proceeding to incubate it with a sample in which the presence and/or the level of a molecule that activates the signal transduction activity of a cell surface protein is sought to be determined. In one preferred embodiment where the cell is irradiated with γ radiation, the cell is preferably maintained and stored at room temperature until use. As the cell is not frozen, a thawing step is unnecessary. After incubation, the level of expression of a reporter gene product, encoded in the reporter gene construct carried by the prepared cell, is determined in the sample. This level of expression as determined by the method according to the present invention is used to then qualitatively determine the presence and/or quantitatively determine the level in a sample of the molecule that activates the signal transduction activity of a cell surface protein.

[0084] A gene reporter assay for Type I interferon is a most preferred embodiment of the present invention. The reporter gene product is preferably firefly luciferase, jellyfish aequorin, or or enhanced green fluorescent protein (EGFP) and is preferably under the control of an interferon sensitive chimeric promoter containing the ISRE from ISG15 and a minimal SV40 promoter. Examples of such reporter gene constructs are presented in Figures 1 and 2. Figure 1 is a schematic representation of a luciferase gene reporter construct in an ISRE-luc vector (SEQ ID NO:1), where the ISRE from ISG15 (SEQ ID NO:5) is positioned at nucleotides 38-97 of SEQ ID NO:1, the SV40 minimal promoter is positioned at nucleotides 103-288 of SEQ ID NO:1, and the coding sequence of the luciferase reporter gene having the amino acid sequence of SEQ ID NO:2 is positioned at nucleotides 328-1980 of SEQ ID NO:1. Similarly, Figure 2 is a schematic representation of a EGFP gene reporter construct in an ISRE-EGFP vector (SEQ ID NO:3), where the ISRE from ISG15 is positioned at nucleotides 30-89 of SEQ ID NO:3, the SV40 minimal promoter is positioned at nucleotides 95-290 of SEQ ID NO:3, and the coding sequence of the EGFP reporter gene having the amino acid sequence of SEQ ID NO:4 is positioned at nucleotides 358-1077 of SEQ ID NO:3.

[0085] As for the cell used in the preferred gene reporter assay for Type I interferon embodiment of the present invention, the cell is preferably a mammalian or avian cell, more preferably a human cell, and most preferably a human promonocytic cell. A preferred human promonocytic cell carrying the ISRE-luc vector containing the luciferase gene reporter construct is a PIL5 cell. The cell is treated to make a commercial cell line that has the commercially desirable properties of a sufficient shelf life for the purpose of the assay and of being a one time use cell that cannot be propagated for possible further use. Preferably, the cell is treated either 1) by irradiating with 6 to 12 Gy of γ radiation, more preferably about 9 Gy, and storage at room temperature for up to 14 days after irradiation or 2) by exposure to an anti-mitotic and pro-apoptotic agent, such as vinblastine, cisplatin, or 5-fluorouracil, most preferably vinblastine, for 10 minutes at 37°C prior to resuspending in a solution containing 40% fetal bovine serum (FBS) and 2.5% DMSO + 10% glycerol and freezing at -80°C.

[0086] In order to optimize the method of obtaining a cell with an indefinite shelf life during frozen storage, but which will die approximately 24 hours after being thawed (once thawed, however, the product has excellent sensitivity, and precision as well as selectivity), the parameters which can be varied in the course of such optimization include:

1) Concentration of FBS. Besides FBS, most any serum could be used as it acts as a toxic sink to protect the cells from toxins, such as while being thawed or while being treated with an anti-mitotic and pro-apoptotic agent. The concentration of FBS can cause the results to vary.

2) Time is a variable. The amount of time of exposure to vinblastine before the cells are centrifuged out and washed to remove vinblastine.

3) The formulation of the vinblastine makes a difference. Presently, soluble vinblastine in a proprietary prebuffered formulation sold by Eli Lilly under the name Velbe in France is preferably used. A different formulation may require slightly different combination of parameters.

4) The concentration of vinblastine.

5) Cell concentration during the vinblastine treatment.

6) The amount of cryopreservative or combination of cryopreservatives.

[0087] All of these parameters can be varied empirically and the results after freezing tested for sensitivity and precision, assuming that the cells stay alive for approximately 24 hours after being thawed. This can be readily determined by one of ordinary skill in the art without undue experimentation, particularly in view of the guidance provided in the experiments shown in Figs. 11-24 for PIL5 cells, in order to arrive at a product having substantially the same sensitivity as the untreated live cells for a period of at least one hour, preferably 8 hours, following thawing but having a viability of no more than 30 days.

[0088] A most preferred embodiment of the present invention is exemplified below in the form of a procedure for conducting a luciferase gene reporter assay for Type I interferon using PIL5 cells treated with the anti-mitotic and pro-apoptotic agent 1 μg/ml vinblastine for 10 minutes at 37°C prior to frozen storage at -20°C and thawing at a later time for purposes of conducting the assay.

PROTOCOL FOR LUCIFERASE GENE REPORTER ASSAY FOR TYPE I INTERFERON USING TREATED PIL5 CELLS

PREPARATION OF MICROTITER ASSAY PLATES

1. PIL5 cells at a concentration of about $2x10^5$ to $7x10^5$ cells/ml in RMPI 1640 medium with 10% fetal bovine serum (FBS) are treated with a fresh solution of 1 μg/ml vinblastine (commercially available from Eli Lilly under the pre-buffered formulation VELBE), diluted from 1 mg/ml in H_2O, for 10 minutes at 37°C in an atmosphere of 5% CO_2 in air. A CO_2 incubator can be used for convenience.
2. The PIL5 cells are centrifuged at 800 x g for 10 minutes at 4°C, and washed once with the same volume of RPMI 1640 medium with 10% FBS to remove the vinblastine.

3. The PIL5 cells are re-suspended at a concentration of $2x10^7$ cells/ml in RMPI 1640 medium with 40% fetal bovine serum (FBS) and 2.5% dimethylsulfoxide + 10% glycerol.
4. The cell suspension is dispensed into the wells of a flat-bottom micro-plate to give 300,000 cells per well (equivalent to 25 μl of cell suspension per well).
5. The micro-plate is frozen at -80°C in an aluminum bag sealed under vacuum with the cover uppermost.
6. The micro-plates can be subsequently stored at -20°C until use.

PREPARATION OF CRYOPRESERVATION AMPOULES/VIALS

1. PIL5 cells at a concentration of about $2x10^5$ to $7x10^5$ cells/ml in RMPI 1640 medium with 10% fetal bovine serum (FBS) are treated with a fresh solution of 1 μg/ml vinblastine (commercially available from Eli Lilly under the prebuffered formulation VELBE), diluted from 1 mg/ml in H_2O for 10 minutes at 37°C in an atmosphere of 5% CO_2 in air. A CO_2 incubator can be used for convenience.
2. The PIL5 cells are centrifuged at 80 x g for 10 minutes at 4°C, and washed once with the same volume of RPMI 1640 medium with 10% FBS to remove the vinblastine.

3. The PIL5 cells are re-suspended at a concentration of 2x10^7 cells/ml in RMPI 1640 medium with 40% fetal bovine serum (FBS) and 2.5% dimethylsulfoxide + 10% glycerol.
4. The cell suspension (1 ml) is dispensed into a cryopreservation vial and frozen at -80%.
5. The cryopreservation vial can be subsequently stored at -20°C until use.

[0089] The assay procedure described below is directed to using the microtiter assay plates prepared above. However, in case the end user elects to obtain the treated PIL5 cells in cryopreservation vials instead of in microtiter plates as predispensed aliquots, the end user may dispense 75 μl of the treated PIL5 cells into each well of a microtiter plate of his/her choice following dilution of the cells 1:6 in RPMI 1640 medium with 10% FBS or into some other receptacles, i.e., Eppendorf microfuge tubes, and conduct the assay in a similar fashion as described below.

ASSAY PROCEDURE

Preparation of Standard Curve

1. Remove protective cover from the sample preparation micro-plate (Plate A).
2. Add 100 μl of the diluent (RPMI 1640 medium without serum) to wells A1 through A6 and B1 through B6 of the sample preparation plate (Plate A).

3. Add 100 μl of the interferon (IFN) reference preparation to wells A1 and B1 of Plate A containing 100 μl of diluent.
4. Carry out serial two-fold dilutions of the IFN reference preparation in Plate A from wells A1 and B1 through wells A6 and B6 using a multi-channel micro-pipette.

Sample preparation

1. Add 100 μl of the diluent to the wells of the sample preparation plate (Plate A) where required.
2. Dilute samples to be tested if the estimated IFN titer of the samples is greater than 100 IU/ml.
3. Dilute samples appropriately in the sample preparation micro-plate (Plate A)

Assay Procedure

1. Rapidly thaw the 96-well PIL5 assay micro-plate (Plate B) on a flat surface with the cover uppermost. A water-bath set at 37°C can be used for convenience.
2. Dry protective cover with a paper hand towel, taking care to maintain the label uppermost.
3. Carefully remove protective cover from the PIL5 assay micro-plate (Plate B) while maintaining the cover uppermost.
4. Add 75 μl of each of the serial two-fold dilutions of the reference preparation to wells A1 and B1 through wells A6

and B6 of the PIL5 assay micro-plate (Plate B) using a multi-channel micro-pipette.

5. Add 75 μl of each undiluted or appropriately diluted sample to the PIL assay micro-plate (Plate A).
6. Incubate the PIL5 assay micro-plate (Plate B) overnight at 37°C in an atmosphere of 5% CO_2 in air. A CO_2 incubator can be used for convenience.
7. Add 100 μl of LUCLIT PLUS (Packard Biosciences, Inc., now part of PerkinElmer Life Sciences, Boston, MA, catalog #6016961) to each well of the PIL5 assay micro-plate (Plate B) using a multi-channel micro-pipette.
8. Determine the luminescence of the samples using a micro-plate luminometer (LUMICOUNT, Packard).

Calculation of International Units

1. Plot standard curve from the mean of the luminescence readings for each dilution of the IFN reference preparation calibrated against the NIH international reference standard for HuIFN-α (G-023-901-527) using Microsoft EXCEL.
2. The equation describing the stand curve is obtained using the EXCEL function "interpolation of the curve" using the option "exponentionel curve". This equation is then used to calculate the IFN titer of each sample expressed in international units (IU).

[0090] While the present invention is directed to commercializing any cell engineered for signal transduction assays, such as those described in U.S. patents 5,436,128 and 5,401,629, the entire contents of which are incorporated herein by reference, there are specific and important utilities for the most preferred embodiments of a gene reporter assay for Type I interferon and the cell used in such an assay. For example, the cell in the interferon gene reporter assay can serve as a surrogate marker for viral infection. It is known that circulating interferon is indicative of a virus infection but circulating interferon does not usually appear in a person who has a bacterial infection. Thus, if a person is suspected of having an infection and one wants to determine whether the infection is viral or bacterial so that treatment can be efficacious and specifically targeted to either a viral or bacterial infection, this interferon gene reporter assay would be extremely useful. In HIV patients, it is known that the circulating interferon level becomes detectable towards the end of the asymptomatic period. Thus, interferon monitoring indicative of disease progression is another utility.

[0091] The cell in the interferon gene reporter assay can also be used as a surrogate marker for detecting viral infections caused by bioterrorism in a susceptible population. If random blood samples are taken in a susceptible population, this simple gene reporter assay allows a relatively fast and inexpensive determination of ubiquitous viral infection which would be an indication of a possible bioterrorism attack.

[0092] Furthermore, rat cell lines, bovine cell lines, avian cell lines, etc., can be created which have the reporter gene under the control of an ISRE in order to determine if animals are infected with a virus, such as to detect bubonic plague in rats, West Nile virus in birds (i.e., crows), hoof and mouth disease in cattle, etc. The presence of West Nile virus in humans can be readily distinguished from most other viral infections because it is one of only a few viral infections that cause a titer of Type I interferons to quickly reach into the tens or hundreds of thousands of units in humans.

[0093] Another utility is to monitor interferon therapy in patients to see how much interferon is actually entering the bloodstream (i.e., from sub-lingual administration) and to determine patient compliance if doses of interferon are self administered.

[0094] As a further utility, the cell and interferon reporter assay can be used to detect autoimmune diseases, such as systemic lupus erythematosus (SLE), Type I diabetes, multiple sclerosis, psoriasis, or rheumatoid arthritis (RA), which are characterized by the presence of interferon in the peripheral circulation or in other body fluids, such as synovial fluids in the case of RA, or to determine the stage of an autoimmune disease. Often these diseases have stages of exacerbation and remission which manifest within the diseased tissue with an increase in circulating interferon immediately prior to an exacerbation phase. Thus, this assay can be used to detect when a patient may be about to enter an exacerbation stage and may offer an opportunity for treatment to prevent or ameliorate the exacerbation stage.

[0095] The present interferon reporter assay recognizes any Type I interferon which binds to a Type I interferon receptor. It is possible, however, to determine which isotype(s) of interferon is being detected by using a parallel monoclonal antibody treatment. Thus, a monoclonal antibody for a specific isotype of interferon will prevent that isotype of interferon from causing signal transduction. If the signal disappears in the presence of antibody, then it is known that the particular interferon being detected is the isotype to which the antibody is specific. The standard activity curve for different isotypes of interferon differ in any given reporter cell line such as for the PIL5 cell line shown in Fig. 3. Such standard activity curves can be generated for each type of interferon in a given reporter cell line. Using such standard curves, one can accurately quantitate the level of interferon present in a sample once it is known what isotype of interferon is being detected.

[0096] Finally, the present invention in general provides a method for commercializing cells having a desired biological activity. This commercial method involves treating cells such that the cells will maintain the desired biological activity for no more than 30 days at a temperature above freezing, and then freezing the treated cells at a temperature and under conditions such that they will resume the required biological activity after thawing. The frozen treated cells, which are available for a limited time but cannot be propagated and maintained indefinitely for multiple uses, are subsequently sold or distributed. The cells are preferably treated with an anti-mitotic and pro-apoptotic agent in a sufficient amount and for a sufficient time such that the treated cells will maintain the desired biological activity for at least 8 hours, but no more than about 30 days at a temperature above freezing. It is preferred that the temperature at which the cells are frozen be about -80°C and that the cells are resuspended in RPMI 1640 medium with 40% FBS containing cryopreservatives, preferably 2.5% DMSO + 10% glycerol, prior to freezing.

[0097] Having now generally described the invention, the same will be more readily understood through reference to the following example which is provided by way of illustration and is not intended to be limiting of the present invention.

EXAMPLE

[0098] The present inventors have overcome the disadvantages of current methods of quantifying interferons by developing a highly sensitive and reproducible method for quantifying IFN activity based on the establishment of a human cell line transfected with the luciferase reporter gene placed under the control of an IFN responsive chimeric promoter. This method of the present invention allows IFN activity to be determined in a few hours rather than in the 3 to 4 days required for a bioassay. Briefly, the interferon stimulatory response element (ISRE) from the ISG15 gene controlling a SV40 minimal promoter was cloned upstream of the luciferase reporter gene in a 5849 bp ISRE-luc vector (Fig. 1; SEQ ID NO:1). Alternatively, the ISRE from the ISG15 gene controlling a SV40 minimal promoter is cloned upstream of an enhanced green florescent protein (EGFP-1) reporter gene in a 4412 bp ISRE-EGFP vector (Fig. 2; SEQ ID NO:3). Human promonocytic U937 cells were transfected with the IFN regulated gene reporter construct and stable transfectants were isolated and cloned. A human cell line PIL5 carrying the luciferase reporter gene under the control of an IFN responsive chimeric promoter was thus established and provides the basis of an assay which allows IFN activity to be determined more rapidly and with greater precision than the standard antiviral bioassay (Lallemand et al., 1996). Table 1 presents the advantages of the luciferase gene reporter assay for interferon using PIL5 cells over the standard antiviral bioassay and Table 2 presents a comparison between the luciferase gene reporter assay for interferon using PIL5 cells and the standard antiviral bioassay on sensitivity to interferon produced as a result of infection by various viruses.

TABLE 1

	GENE REPORTER ASSAY	BIOASSAY
Sensitivity	0.1 IU	1 to 5 IU
Response time	4-8 hrs	3 to 4 days
Use	General	Restricted
Cost	Reagent and labour costs low > 90% gross margin	High reagent costs Labour intensive

TABLE 2

Gene Reporter Assay IU/ml	Bioassay IU/ml	Etiology
>50	25	Adenovirus
17	6	IFN treatment
>50	37	Influenza B
>50	25	Para Influenza I
30	9	HIV
45	12	HIV
>50	37	Rotavirus
2	Undetected	HCV
4	Undetected	HCV

[0099] The gene reporter assay using PIL5 cells is highly sensitive (less than 1.0 IU/ml of IFNα or IFNβ can be detected routinely), reproducible (standard error +/- 10%), and can detect IFN activity over a wide range of concentrations (0.1 to 100 IU/ml). The method is also highly specific and can for example even detect low levels of Type I IFNs (IFNα or IFNβ) in the presence of high levels of IFNγ which is not possible using a conventional antiviral bioassay. The method is ideally suited for the determination of IFN activity in biological fluids such as human serum, cerebrospinal fluid, or urine as the method is less subject to non-specific interference at low dilutions than the conventional anti-viral bioassay. Human serum and other biological fluids often contain non-specific inhibitors of virus-replication unrelated to IFN which can affect virus replication at low dilutions giving rise to false positives.

[00100] The PIL5 gene-reporter IFN assay recognizes any Type I interferon which binds to a Type I interferon receptor. It also provides a means of distinguishing between one IFNα sub-type and another due to the ability to detect differences in the dose response (standard) curves characteristic of individual IFNα sub-types. This is of considerable value in distinguishing between different virus infections. Paramyxo viruses such as Sendai induce mainly IFNα1, IFNα2, and IFNβ, while Lentiviruses such as HIV-1 induces mainly IFNα5 (Lallemand et al., 1996). Standard curves of luciferase activity in PIL5 cells in the presence of different amounts of each of the various isotypes of interferon is presented in Figure 3.

[00101] Thus, a method has been developed for the determination of IFN levels in biological fluids which has been used experimentally to analyze numerous clinical samples from virus

infected individuals. The method is rapid, inexpensive, robust, does not require specialized personnel or equipment, and is readily automated. The PIL5 gene-reporter assay is based, however, on the use of live cells which limits commercialization in its present form, due to a severely limited shelf life and the ability of a customer to retain and cultivate the PIL5 cell line thereby obviating the necessity to purchase further kits. The experiments presented below were conducted to develop a modified form of the PIL5 gene reporter assay amenable to commercialization in a kit format, where the PIL5 cells are treated so as to have a sufficiently long shelf life as part of a commercial assay, and whereupon at the end of its useful shelf life or at the end of its use in a PIL5 gene reporter assay, the treated PIL5 cells undergo cellular death such as by apoptosis. The experimental approach adopted as described below is based on the use of fractionated doses of γ irradiation or on the use of anti-mitotic and pro-apoptotic agents to prevent cell multiplication and to induce delayed cellular death (i.e., apoptosis) while retaining a functional IFN signal transduction pathway.

Effect of γ irradiation on IFN sensitivity

[00102] PIL5 cells were subjected to increasing doses of γ irradiation (0.32 to 25 Gy) and then tested at various times thereafter for their ability to detect increasing doses of Type I

interferons. The results of preliminary experiments indicated that PIL5 cells retain full IFN sensitivity when incubated at 37°C for up to 4 days after doses of γ irradiation between 0.32 and 12 Gy (Figure 4), and that apoptosis is induced in 100 % of the cell population exposed to doses of γ irradiation of 6 to 25 Gy (Table 3). Thus, these results validated the experimental approach adopted and suggested that the potential shelf life of the PIL5 gene reporter assay could be extended significantly beyond 4 days by exposing cells to fractionated doses of γ irradiation within the range of 6 to 12 Gy while inducing 100% (or nearly 100% for 6 Gy) apoptosis of the cell population (Figure 5 and Table 3).

TABLE 3

Percentage of Cells Viable after Irradiation

Time After Irradiation	25 Gy	12 Gy	6 Gy	3 Gy	1,5 Gy	0,75 Gy	0,32 Gy	Control
Day 1	100	100	100	100	100	100	100	100
Day 2	60	100	100	100	100	100	100	100
Day 3	40	80	100	100	100	100	100	100
Day 4	30	50	80	100	100	100	100	100
Day 8	0	0	0	50	50	100	100	100
Day 21	0	0	100	100	100	100	100	100

Effect of temperature on IFN sensitivity

[00103] The results of a series of experiments have established that PIL5 cells can indeed be stored in RPMI 1640 medium with 10% fetal calf serum for up to 13 days at room temperature following exposure to a dose of γ irradiation of 6 Gy and still retain full IFN sensitivity (Figure 6). That is provided that the PIL5 cells are subsequently incubated with the test samples or IFN standard at 37°C, prior to assaying luciferase activity. In contrast, it has been unexpectedly shown that incubation of PIL5 cells at 4°C in RPMI 1640 medium with 10% fetal calf serum following exposure of the cells to a dose of γ irradiation of 6 Gy induced cell death. This resulted in a marked loss of IFN sensitivity after only 8 days incubation at 4°C (Figure 7).

Effect of serum concentration on IFN sensitivity

[00104] In an additional series of experiments, the serum content of the culture medium was varied between 1 and 10% in order to modulate exposure of cells to serum growth factors, the signalling of which is known to counter the induction of apoptosis by γ irradiation. The results of these experiments showed that induction of apoptosis in PIL5 cells is more rapid when the cells are incubated in culture medium containing 1% fetal bovine serum (indicated as FBS in Fig. 8) serum than in culture medium containing 10% serum (Figure 8). Furthermore,

increasing the serum concentration from 10 to 20% did not affect significantly cell viability.

Effect of cell concentration on IFN sensitivity

[00105] It was expected that the sensitivity of the present PIL5 IFN gene reporter assay could be increased significantly simply by increasing the number of PIL5 cells used in the assay. Such a super high sensitivity (SHS) version of the PIL5 gene reporter assay would find wide application for the determination of very low levels of IFN in biological fluids such as cerebro-spinal fluid. Thus, PIL5 cells were incubated at concentrations of 0.1 to 1.0 x 10^6 cells/ml at room temperature in culture medium containing 10% fetal bovine serum following exposure to a dose of 6 Gy of γ irradiation and then tested at various times thereafter for their ability to detect increasing doses of IFN α. The results of these experiments showed that, although interferon sensitivity was indeed increased initially, incubation of PIL5 cells at high cell density induced cellular death more rapidly than at a concentration of 0.1 x 10^6 cells/ml, most probably due to depletion of growth factors in the culture medium or production of toxic metabolites (Figure 9).

Effect of the inhibitors of apoptosis phenylarsine oxide and aurintricarboxylic acid on IFN sensitivity

[00106] In an attempt to extend the shelf-life of the PIL5 IFN assay, PIL5 cells were subjected to 6 Gy of γ irradiation and

then treated with increasing concentrations (0, 0.1, 1.0, 10, and 100 μM) of aurintricarboxylic acid or phenylarsine in order to modulate the induction of apoptosis. The cells were then tested at different times thereafter (0, 5, 10, 15, 20 days etc) for IFN sensitivity.

[00107] The results of these studies showed that the addition of increasing concentrations of aurintricarboxylic acid did not extend the shelf life of PIL-5 gene reporter following exposure of cells to a dose of 6 Gy of γ irradiation (Figure 10).

Effect of treatment with an anti-mitotic agent and subsequent freezing on IFN sensitivity

[00108] PIL5 cells were suspended at a concentration of $2x10^6$ cells/ml in RPMI 1640 medium plus 10% fetal bovine serum (FBS) and incubated at 37°C for 1.0 hour in the presence of 100 μM vinblastine. The cells were then centrifuged at 800xg for 10 minutes, washed once with the same volume of RPMI 1640 medium with 10% FBS to remove vinblastine, and re-suspended in RPMI 1640 medium plus 40% fetal bovine serum, 10% glycerol and 2.5% dimethylsulfoxide (DMSO). The cell suspension (10^5 cells in a volume of 25 μl) is then distributed into the wells of a micro-titer plate (CulturePlate-96, Packard Biosciences, Inc., now part of PerkinElmer Life Sciences, Boston, MA) and stored at -20°C until use. The micro-titer plates containing the frozen cells are then removed from the -20°C freezer, thawed rapidly,

preferably using a waterbath preset at 37°C, and 75 µl of the sample to be tested, or the IFN standard without serum, or negative control sample without serum, is added to a well of the micro-titer plate and incubated overnight at 37°C. One hundred micro-liters of LUCLIT PLUS (Packard Biosciences, Inc., Catalog #6016961, U.S. Patent Nos. 5,283,179; 5,641,641; 5,650,289; and 5,814,471) or a similar reagent, which contains luciferin (D-(-)-2-(6'-hydroxy-2'-benzothiazolyl)-Δ^2-thiazoline-4-carboxylic acid), ATP, buffer, Mg^{+2}, and preferably coenzyme A fr enhancing the activity of luciferase and a thiol reagent/sulfhydryl compound for stabilizing luciferase, is added to each well of the micro-titer plate and the samples are read on a plate reading luminometer (LUMICOUNT, Packard BioSciences, Inc.). PIL5 cells were found to retain full IFN sensitivity at one month after treatment with 100 µM vinblastine and freezing at -20°C (Figure 11). The results of this study have established a method for the development of the PIL5 IFN gene reporter assay in a commercially viable kit format with a current shelf-life of at least one month at -20°C without loss of IFN sensitivity.

[00109] Studies on the effects of a variety of parameters, i.e., concentration of vinblastine, time of treatment with vinblastine, fetal bovine serum (FBS) concentration, glycerol concentration in the presence or absence of DMSO, SMSO concentration, cell density, on interferon sensitivity of frozen

cells were conducted to determine optimal conditions. See Figs. 12-20. It may not be apparent from the results shown in Fig. 12 why vinblastine instead of 5-FU was chosen as the more preferred anti-mitotic and pro-apoptotic agent for treatment of PIL5 cells prior to freezing. The reason is that although 5-FU was superior to vinblastine in terms of maintaining interferon sensitivity, 5-FU was not found to be sufficiently effective in preventing cell replication at the doses tested.

[00110] Figures 21 and 22 show the effect of cell number on interferon sensitivity of the treated PIL5 cells following freezing, and Figure 23 is a study on the effect of freezing in either a microtiter plate or in a cryopreservation ampoule/vial on interferon sensitivity of thawed treated PIL5 cells. A titration curve of a standard interferon preparation on treated PIL5 cells following freezing is presented in Fig. 24.

[00111] Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

[00112] While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the

inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

[00113] All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

[00114] Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

[00115] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific

embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

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